Title	Adrenomedullin facilitates calcium channel currents in osteoblasts.
Author(s)	Tazaki, M; Endoh, T; Kobayashi, H; Nobushima, H; Shibukawa, Y; Tsumura, M; Sato, M; Ubaidus, S; Sueishi, K
Journal	Bulletin of Tokyo Dental College, 53(4): 203-206
URL	http://hdl.handle.net/10130/3271
Right	

Short Communication

Adrenomedullin Facilitates Calcium Channel Currents in Osteoblasts

Masakazu Tazaki, Takayuki Endoh, Hiroshi Kobayashi*, Hiromi Nobushima, Yoshiyuki Shibukawa, Maki Tsumura, Masaki Sato, Sobhan Ubaidus and Kenji Sueishi*

Department of Physiology, Tokyo Dental College,
1-2-2 Masago, Mihama-ku, Chiba 261-8502, Japan
* Department of Orthodontics, Tokyo Dental College,
1-2-2 Masago, Mihama-ku, Chiba 261-8502, Japan

Abstract

Osteoblasts play a major role in bone formation. Osteoblasts employ intracellular Ca^{2+} as a second messenger to modulate hormonal responses and a cofactor for bone mineralization. Adrenomedullin (ADM) promotes osteoblast growth and proliferation, inducing an increase in bone mass. Voltage-dependent Ca^{2+} channels (VDCCs) mediate the influx of Ca^{2+} in response to membrane depolarization. Voltage-dependent Ca^{2+} channels serve as crucial mediators of many Ca^{2+}-dependent functions, including growth of bone and regulation of proliferation. The purpose of this study was to investigate the effects of ADM on VDCC currents in osteoblasts using a patch-clamp recording method. To our knowledge, the data presented here demonstrate for the first time that ADM facilitates VDCCs in osteoblasts.

Key words: Adrenomedullin — Osteoblast — Voltage-dependent Ca^{2+} channels — Patch clamp experiment

Introduction

In cells of osteoblastic lineage, intracellular Ca^{2+} signals coordinate cell behavior, which translates into systemic control of Ca^{2+} homeostasis and maintenance of normal bone mineral density. Adrenomedullin (ADM) is a 52-amino-acid peptide originally isolated from a human pheochromocytoma[12]. It is structurally and functionally related to calcitonin gene-related peptide (CGRP) and belongs to the amylin peptide family[14]. Dorit *et al.* demonstrated that ADM receptors are expressed in osteoblasts[6]. Several studies have shown that ADM stimulate osteoblasts proliferation[2,3,9]. Voltage-dependent Ca^{2+} channels (VDCCs) serve as crucial mediators of membrane excitability[15] and many Ca^{2+}-dependent functions such as growth of bone[7], regulation of proliferation[13], enzyme activity[17] and gene expression[16]. Modulation of VDCCs by CGRP and ADM in various types of cells has been described previously[8,11,19]. However, the effect of ADM on VDCCs in osteoblasts remains to

be clarified. Therefore, the purpose of this study was to investigate the effects of ADM on VDCC currents (I_{Ca}) in osteoblasts.

Materials and Methods

Murine osteoblastic MC3T3-E1 cells were purchased from Summit Pharmaceuticals International Corporation (Tokyo, Japan). Cells were cultured at 37°C in a 5%(v/v) CO_2 atmosphere with α-modified minimal essential medium (α-MEM; Gibco BRL, Grand Island, NY, U.S.A.). Unless otherwise specified, the medium contained 10%(v/v) heat-inactivated fetal bovine serum, 100 U/ml penicillin and 100 mg/ml streptomycin. Cell culture medium was changed every 2–3 days. For patch-clamp experiments, cells were harvested using a 0.05% trypsin/0.02% EDTA solution, when cells reached confluence. Cells were plated at very low density in 35 mm tissue culture dishes. Prior to recordings, the cells were washed at least three times with Krebs solution of the following composition (in mM): 136 NaCl; 5 KCl; 2.5 $CaCl_2$; 0.5 $MgCl_2$; 10.9 glucose; 11.9 $NaHCO_3$ and 1.1 NaH_2PO_4. The pH was 7.3–7.4. Cell culture reagents were purchased from Sigma (Tokyo, Japan).

Voltage-clamp recordings were obtained using whole-cell configuration patch-clamp technique[10]. Fabricated recording pipettes (2–3 MΩ) were filled with internal solution of the following composition (in mM): 150 CsCl; 5 EGTA; 10 D-glucose and 10 HEPES. The pH was adjusted to 7.3 with CsOH. After the formation of a giga seal, in order to record I_{Ca} carried by Ba^{2+} (I_{Ba}), the extracellular solution was replaced changing Krebs solution for a solution containing the following (in mM): 115 $BaCl_2$ and 20 HEPES. The pH was adjusted to 7.4 with tetraethylammonium hydroxide (TEA-OH). Command voltage protocols were generated with the computer software pCLAMP version 10 (Axon Instruments, Union City, CA, U.S.A.) and transformed to an analogue signal using the DigiData 1440A interface (Axon Instruments). The command pulses were applied to the cells through an L/M-EPC7 amplifier (HEKA Elektronik, Lambrecht, Germany). The currents were recorded with an amplifier and the computer software pCLAMP10 acquisition system. Access resistance ($<$15 MΩ) was determined by transient responses to voltage commands. Adrenomedullin was purchased from Sigma.

Results

The properties of VDCCs in osteoblasts using current-voltage relationships have been demonstrated previously[18]. Osteoblasts have L-type VDCCs, which are fully activated at a test potential ($= -10$ mV) from a holding potential ($= -80$ mV). Therefore, in this study, full activation of I_{Ba} was obtained by applying a test pulse from a holding potential of -80 mV in depolarizing voltage steps of -10 mV evoked every 20 sec.

Representative examples of superimposed I_{Ba} traces in the absence or presence of 1 μM ADM are shown in Figs. 1A and B. As shown in Figs. 1A and B, application of 1 μM ADM rapidly and reversibly facilitated I_{Ba} from -49 pA to -127 pA (159% facilitation) in this cell. Representative examples of superimposed I_{Ba} traces in the absence or presence of 0.1 μM ADM are shown in Figs. 1C and D. As shown in Figs. 1C and D, application of 0.1 μM ADM rapidly and reversibly facilitated I_{Ba} from -78 pA to -93 pA (19% facilitation) in this cell.

Discussion

The results of this study showed that ADM facilitates VDCCs in osteoblasts.

Cornish *et al.* has suggested that ADM stimulate osteoblast proliferation and increases intracellular Ca^{2+} levels ($[Ca^{2+}]_i$)[4]. It is possible that ADM-induced proliferation is due to an increase in $[Ca^{2+}]_i$ by facilitation of VDCCs.

There are several mechanisms of VDCCs facilitation[5]. L-type VDCCs facilitation can result from a strong conditioning depolari-

A

B

ADM (1 μM)
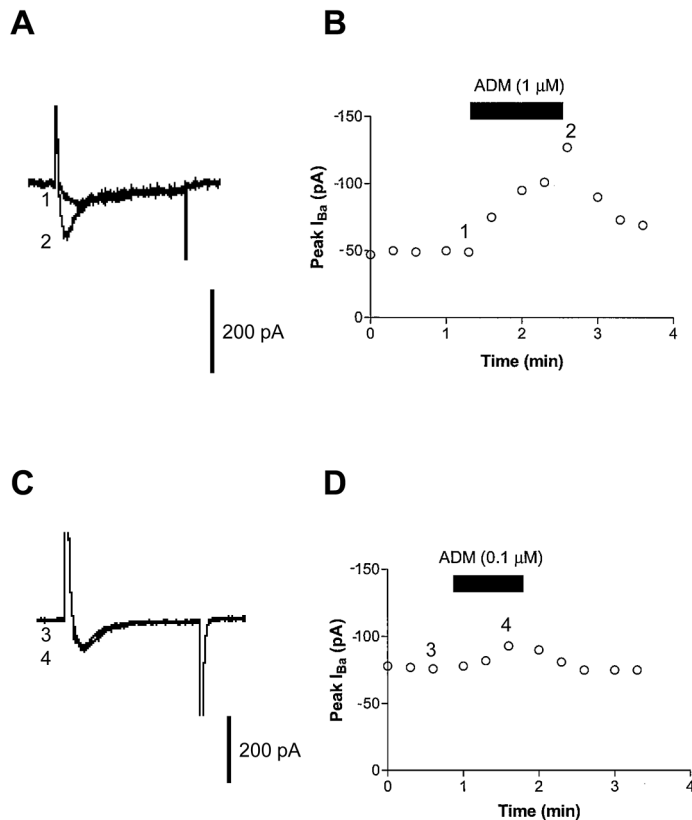
C

D

ADM (0.1 μM)

200 pA

200 pA

Fig. 1 A: Typical superimposed I$_{Ba}$ traces according to time course graph B. Panel A shows corresponding current traces indicated by numbers (1 and 2) in B. I$_{Ba}$ was evoked from holding potential of -80 mV in 100-msec voltage steps to -10 mV at 20-sec intervals. B: Typical time course of ADM-induced modulation of I$_{Ba}$. ADM (1 μM) was bath-applied during times indicated by black bar. C: Typical superimposed I$_{Ba}$ traces according to time course graph D. Panel C shows corresponding current traces indicated by numbers (3 and 4) in D. D: Typical time course of ADM-induced modulation of I$_{Ba}$. ADM (0.1 μM) was bath-applied during times indicated by black bar.

zation that recruits silent channels[1]. Alternatively, L-type VDCCs can be facilitated by protein kinases[14]. L-type VDCCs possess several consensus protein kinase A (PKA) and protein kinase C (PKC) phosphorylation sites and physiological studies have demonstrated channel facilitation by these enzymes[14]. We previously demonstrated that ADM facilitates VDCCs mediated by PKA in submandibular ganglion[8]. We have also demonstrated that ADM facilitates VDCCs involving mitogen-activated protein kinase in nucleus tractus solitarius[11]. It is possible that ADM could play a role in therapy for osteoporosis. Therefore, ADM receptor's intracellular pathways in

osteoblasts should be investigated in further study.

References

1) Artelejo CR, Ariano MA, Perlman RL, Fox AP (1990) Activation of facilitation calcium channels in chromaffin cells by D1 dopamine receptors through a cAMP/protein kinase A-dependent mechanism. Nature 348:239–242.
2) Cornish J, Callon KE, Bava U, Coy DH, Mulvey TB, Murrey MA, Cooper GJ, Reid IR (2001) Systemic administration of adrenomedullin (27–52) increases bone volume and strength in male mice. Endocrinology 170:251–257.

3) Cornish J, Callon KE, Coy DH, Jiang NY, Xiao L, Cooper GJ, Reid IR (1997) Adrenomedullin is a potent stimulator of osteoblastic activity *in vitro* and *in vivo*. Am J Physiol 273: E1113–E1120.

4) Cornish J, Naot D, Reid IR (2003) Adrenomedullin—a regulator of bone formation. Regul Pept 112:79–86.

5) Dolphin AC (1996) Facilitation of Ca^{2+} current in excitable cell. Trends Neurosci 19:35–43.

6) Dorit N, Karen EC, Andrew G, Garth JSC, Ian RR, Jullian C (2001) A potential role for adrenomedullin as a local regulator of bone growth. Endocrinology 142:1849–1857.

7) Duriez J, Flautre B, Blary MC, Hardouin P (1993) Effects of the calcium channel blocker nifedipine on epiphyseal growth plate and bone turnover: a study in rabbit. Calcif Tissue Int 52:120–124.

8) Endoh T, Shibukawa Y, Tsumura M, Ichikawa H, Tazaki M, Inoue T (2011) Calcitonin gene-related peptide- and adrenomedullin-induced facilitation of calcium current in submandibular ganglion. Arch Oral Biol 56:187–193.

9) Hamada H, Kitamura K, Chosa E, Eto T, Tajima N (2002) Adrenomedullin stimulates the growth of cultured normal human osteoblasts as an autocrine/paracrine regulator. Peptides 23:2163–2168.

10) Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pfügers Arch 391:85–100.

11) Hosokawa S, Endoh T, Shibukawa Y, Tsumura M, Ichikawa H, Tazaki M, Furusawa M (2010) Calcitonin gene-related peptide- and adrenomedullin-induced facilitation of calcium current by different signal pathways in nucleus tractus solitarius. Brain Res 1327: 47–55.

12) Kitamura K, Kangawa K, Kawamoto M, Ichiki Y, Nakamura S, Matsuo H, Eto T (1993) Adrenomedullin: A novel hypotensive peptide isolated from human phenochromocytoma. Biochem Biophys Res Commun 192:553–560.

13) Loza J, Stephan E, Dolce C, Dziak R, Simasko S (1994) Calcium currents in osteoblastic cells: dependence upon cellular growth stage. Calcif Tissue Int 55:128–133.

14) McDonald TF, Pelzer S, Trautwein W, Pelzer DJ (1994) Regulation and modulation of calcium channels in cardiac, skeletal and smooth muscle cells. Physiol Rev 74:365–507.

15) Miller RJ (1987) Multiple calcium channels and neuronal function. Science 235:46–52.

16) Murphy TH, Worley PH, Baraban JM (1991) L-type voltage-sensitive calcium channels mediate synaptic activation of immediate early genes. Neuron 7:625–635.

17) Reuter H (1983) Calcium channel modulation by neurotransmitters, enzymes and drugs. Nature 301:569–574.

18) Uchida Y, Endoh T, Tazaki M, Sueishi K (2012) Chronic bradykinin treatment alters $1\alpha,25$-dihydroxyvitamin D_3-induced calcium current modulation in pre-osteoblasts. Cell Calcium 51:383–392.

19) Zhang X-H, Li G-R, Bourreau J-P (2007) The effect of adrenomedullin on the L-type calcium current in myocytes from septic shock rats: signaling pathway. Am J Physiol Heart Circ Physiol 293:H2888–H2893.

Reprint requests to:
        Dr. Takayuki Endoh
        Department of Physiology,
        Tokyo Dental College,
        1-2-2 Masago, Mihama-ku,
        Chiba 261-8502, Japan